2449-Pos Board B219
Effects of Covalent Modifications on the Structure and Assembly of Nucleosomes
Ju Yeon Lee, Sijie Wei, **Tae-Hee Lee**.
The Pennsylvania State University, University Park, PA, USA.
We studied the structure of nucleosomes, the assembly of nucleosomes and how these properties are altered by DNA methylation and histone acetylation based on single molecule and ensemble fluorescence measurements. Our study revealed that a compact and rigid nucleosome structure is induced by CpG methylation in both internal and terminal regions of nucleosomal DNA. Real-time monitoring of nucleosome assembly with NAP1 revealed that there are at least 3 stable intermediate states during the assembly. The kinetic stabilities of the intermediate states are significantly elevated upon CpG methylation. These results suggest that CpG methylation stabilizes the nucleosome structure and inhibits the disassembly by destabilizing the transition states. We also characterized effects of histone acetylation by Piccolo NuA4 on the structure of a nucleosome and dinucleosomes. Upon the acetylation, we observed directional unwrapping of nucleosomal DNA that accompanies a topology change. We proposed a structural model for a dinucleosome in chromatin based on the structure of a dinucleosome spontaneously formed by two mononucleosomes in solution, which depends strongly on Mg2+ concentration and histone acetylation state. Mainly rendered by single molecule observations, these results suggest that structural changes of nucleosomes induced upon DNA methylation and histone acetylation may contribute to the regulation of genome activities.

2450-Pos Board B220
The Effectcs of Histone H4 Acetylations in Nucleosome-Nucleosome Interactions and on Chromatin Folding and Fibre-Fibre Association
Lars Nordenskiöld, Nikolay Korolev, Abdollah Allahverdi, Ying Liu, Renliang Yang, Yanping Fan, Chuan-Fa Liu.
Nanyang Technological University, Singapore, Singapore.
We developed novel semi-synthetic methods for the preparation of precisely modified N-terminal histone tails and investigated the folding and self-association of recombinant nucleosome arrays using a range of biophysical methods. These arrays contained acetylations at positions 5, 8, 12, and 16 of histone H4 and the condensation of this chromatin system, induced by cations like Mg^{2+} was systematically studied. Additionally, the effects of the combination of acetylations and H4 tail methylations, linker histone H1 and nucleosome repate length is also studied. It was found that: i) The single clean and complete H4-K16 acetylation is sufficient to severely antagonize array folding, while acetylations at positions 5, 8 and 12 together only have a moderate effect that acts additively when combined with H4-K16Ac. ii) Inter-array self-association is unspecifically electrostatically controlled by the charge reduction effect of acetylation and follows polyelectrolyte behaviour. iii) The cation K^+, at physiological concentrations, impede full nucleosome array folding almost to the same extent as H4-K16Ac. The observations suggests that nucleosome-nucleosome stacking, required for full folding, is mediated by H4K16 binding to a site on the H2B histone, which is disrupted by acetylation or the presence of K^+ ions. The nature of the nucleosome-nucleosome interactions and role of tails and H4 acetylations were further investigated by studying solutions of mononucleosomes (NCP) with tail acetylations or alternatively, devoid of tails, using synchrotron X-ray scattering. Contrary to array folding, close nucleosome-nucleosome stacking is observed for NCPs with acetylated H4 histone tails, as shown by the formation of an hexagonal columnar phase of ordered mononuclesomes, induced by cations. Computer simulations with a detailed coarse grained model indicate that aggregation to stacked NCPs can be completely driven by electrostatic interactions.

2451-Pos Board B221
Sequence-Specific Asymmetric Binding of Linker Histone to Nucleosome
Victor Zhurkin[1], Tatiana Nikitina[1], Difei Wang[1], Feng Cui[1], Michael Gomberg[2], Sergei Grigoryev[2].
[1]NIH, Bethesda, MD, USA, [2]Pennsylvania State University College of Medicine, Hershey, PA, USA.
Linker histones (LHs) are critical for the formation of higher-order chromatin structure. Earlier studies showed that LHs exhibit a general preference for AT-rich DNA linkers. To account for this preference, we proposed that the asymmetric LH binding to nucleosome is stabilized by hydrophobic interaction between the 'wing' domain of LH and the thymine methyl groups in the major groove of linker DNA (Cui and Zhurkin, Nucl. Acids Res. 2009). To test this hypothesis, we prepared several constructs (based on the strongly positioned nucleosome '601' designed by Lowary and Widom) with variable DNA linkers. The AT-rich constructs contained the TTT:AAA motif at the entry-exit point, whereas the GC-rich constructs had no A:T pairs at the nucleosome boundary.
The band-shift experiments (6% PAGE) showed that the LH affinity to nucleosome is ~1.7 times higher in the case of the AT-rich linker. Although this difference is not very significant, it was reliably reproduced. (The apparent *K*d = 20 ± 3 nM [LH] for the AT-rich construct and 35 ± 5 nM for the GC-rich construct. Note that these *K*d values reflect a high amount, 50 nM, of the wild type 601 construct used as a 'competitor' during gel electrophoresis.)
In the second set of experiments we measured the LH-induced protection of linker DNA from the EcoRV cleavage. The EcoRV site, GATATC, was incorporated in the linker, 6-11 bp away from the entry-exit point of nucleosome (where LH is predicted to bind the DNA minor groove). In agreement with our model, the EcoRV cleavage rate of the AT-rich linker constitutes ~60% of the corresponding rate for the GC-rich linker. The results are consistent with our hypothesis on the asymmetric binding of LH to the AT-rich linker DNA (which is tacitly ignored in the other models).

2452-Pos Board B222
DNA Sequence Dependent Affinities for Nucleosome Formation Calculated by Thermodynamic Integration
Gregory G. Putzel, Nimesh Jain, Igal Szleifer.
Northwestern University, Evanston, IL, USA.
The positioning of nucleosomes within the genome is important in regulating the expression of genes. We calculate the differences in free energy of nucleosome formation between different DNA sequences by performing thermodynamic integration on a coarse-grained model of a nucleosome. We find small differences in free energies of nucleosome formation between different DNA sequences, consistent with the fact that the effects of intrinsic DNA sequence preferences for nucleosome formation are present but not dominant in vivo. We also determine how the presence of molecular crowders affects these free energy differences.

2453-Pos Board B223
Single-Molecule Twist and Stretch of Mononucleosome using Optical Torque Wrench
Jen-Chien Chang[1], Michel de Messieres[1], Olga I. Kulaeva[2], Vasily M. Studitsky[2], Arthur La Porta[1].
[1]University of Maryland, College Park, MD, USA, [2]University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School, Piscataway, NJ, USA.
The fundamental chromatin packing unit in eukaryotes is the nucleosome, where ~147 base pairs of DNA are wrapped in ~1.7 turns around a core histone octamer. A crucial question in biology is to explain how proteins are able to access DNA which is tightly bound in. For example, RNA polymerase must navigate through the nucleosome while transcribing DNA. Hence, the DNA-histone interactions play a key role in gene regulation. Single-molecule force spectroscopy is a powerful tool to probe this system. Prior studies have exerted linear tension to stretch both chromatin fibers and mononucleosome molecules, which gave information on the nature of the free-energy barrier for a particular disruption pathway. Theoretical studies have suggested that the disruption pathway may be strongly sensitive to the torsional loading of the nucleosome. This is of interest because helicases, polymerases, or other motor proteins may use a combination of force and torque to disrupt chromatin. Here we simultaneously apply force and torque to a mononucleosome structure using an optical torque wrench. By determining the influence of supercoiling density on the disruption barrier we obtain more detailed information on nucleosome unwrapping dynamics.

2454-Pos Board B224
Single Molecule Force Spectroscopy Reveals a Left Handed Helical Folding for the 30 nm Chromatin Fiber
He Meng[1], Thijn van der Heijden[1], Fabrizio Martino[2], Daniela Rhodes[2], John van Noort[1].
[1]Leiden University, Leiden, Netherlands, [2]University of Cambridge, Cambridge, United Kingdom.
In eukaryotic cells, genetic and epigenetic information is stored in DNA and histone proteins which together form compact chromatin fibers. The structure of chromatin fibers has been heavily debated, but experiments in vitro clearly show folding of arrays of nucleosomes into 30 nm thick fibers. The stability of these fibers and their nucleosomes has been implicated to depend on both force and torque. In previous force spectroscopy experiments, we showed